Lecture V

Organization and expression of immunoglobulin genes

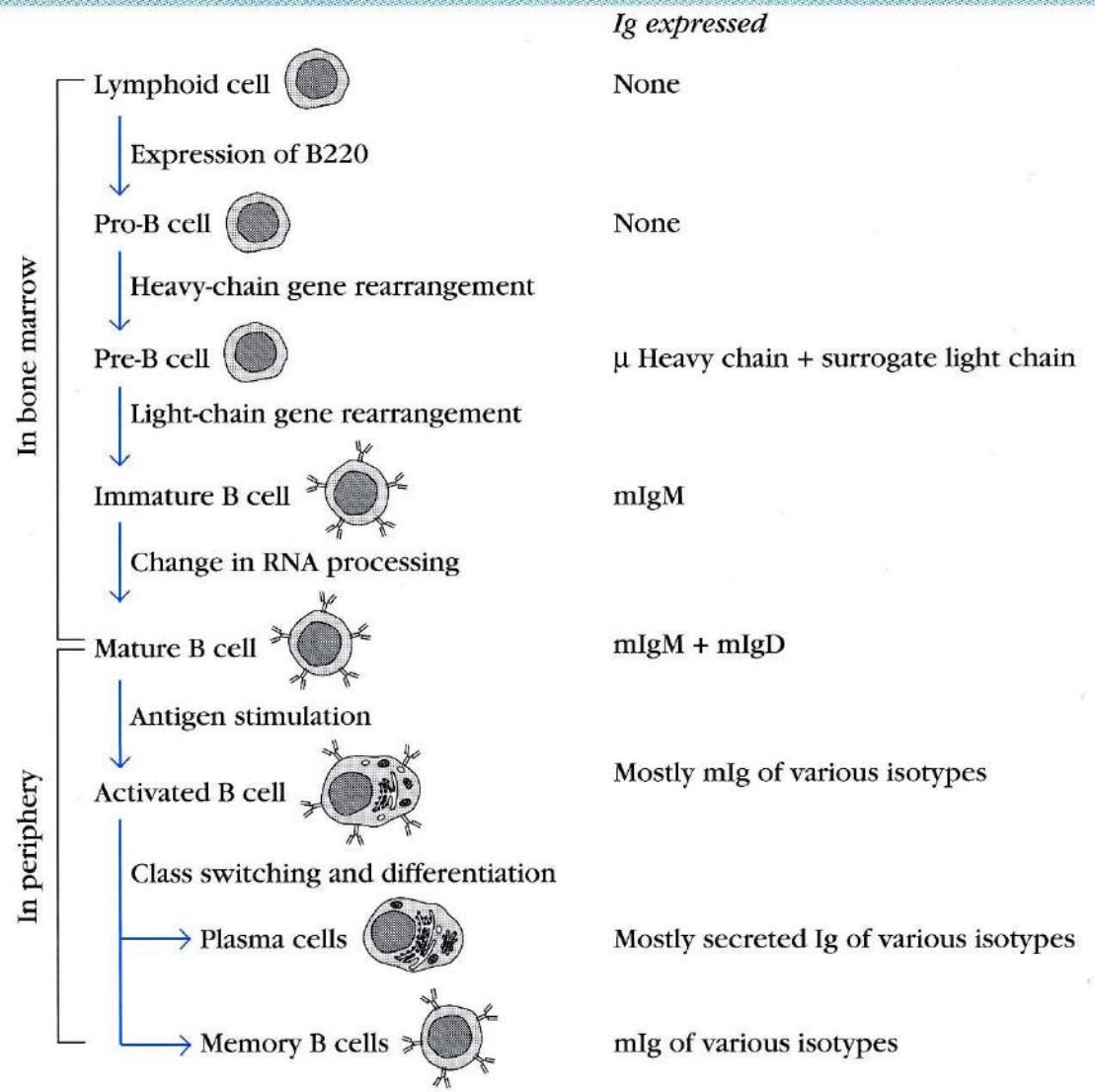


FIGURE 7-1

Overview of B-cell development. The events that occur during maturation in the bone marrow do not require antigen, whereas activation and differentiation of mature B cells in peripheral lymphoid organs require antigen. mIgM, mIgD, and mIgG refer to membrane-associated Igs.

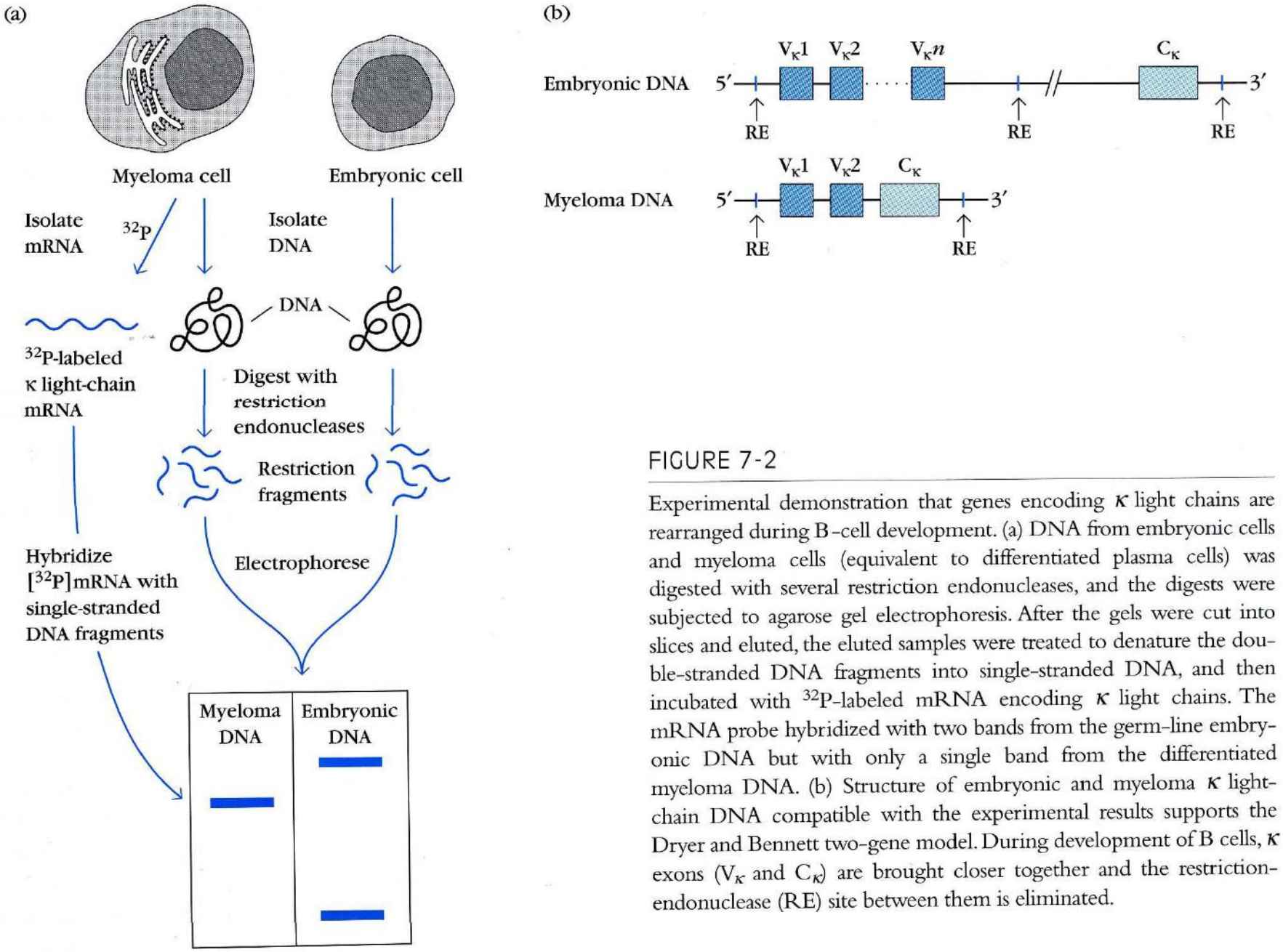


FIGURE 7-2

Experimental demonstration that genes encoding κ light chains are rearranged during B-cell development. (a) DNA from embryonic cells and myeloma cells (equivalent to differentiated plasma cells) was digested with several restriction endonucleases, and the digests were subjected to agarose gel electrophoresis. After the gels were cut into slices and eluted, the eluted samples were treated to denature the double-stranded DNA fragments into single-stranded DNA, and then incubated with ^{32}P-labeled mRNA encoding κ light chains. The mRNA probe hybridized with two bands from the germ-line embryonic DNA but with only a single band from the differentiated myeloma DNA. (b) Structure of embryonic and myeloma κ light-chain DNA compatible with the experimental results supports the Dryer and Bennett two-gene model. During development of B cells, κ exons (V_κ and C_κ) are brought closer together and the restriction-endonuclease (RE) site between them is eliminated.

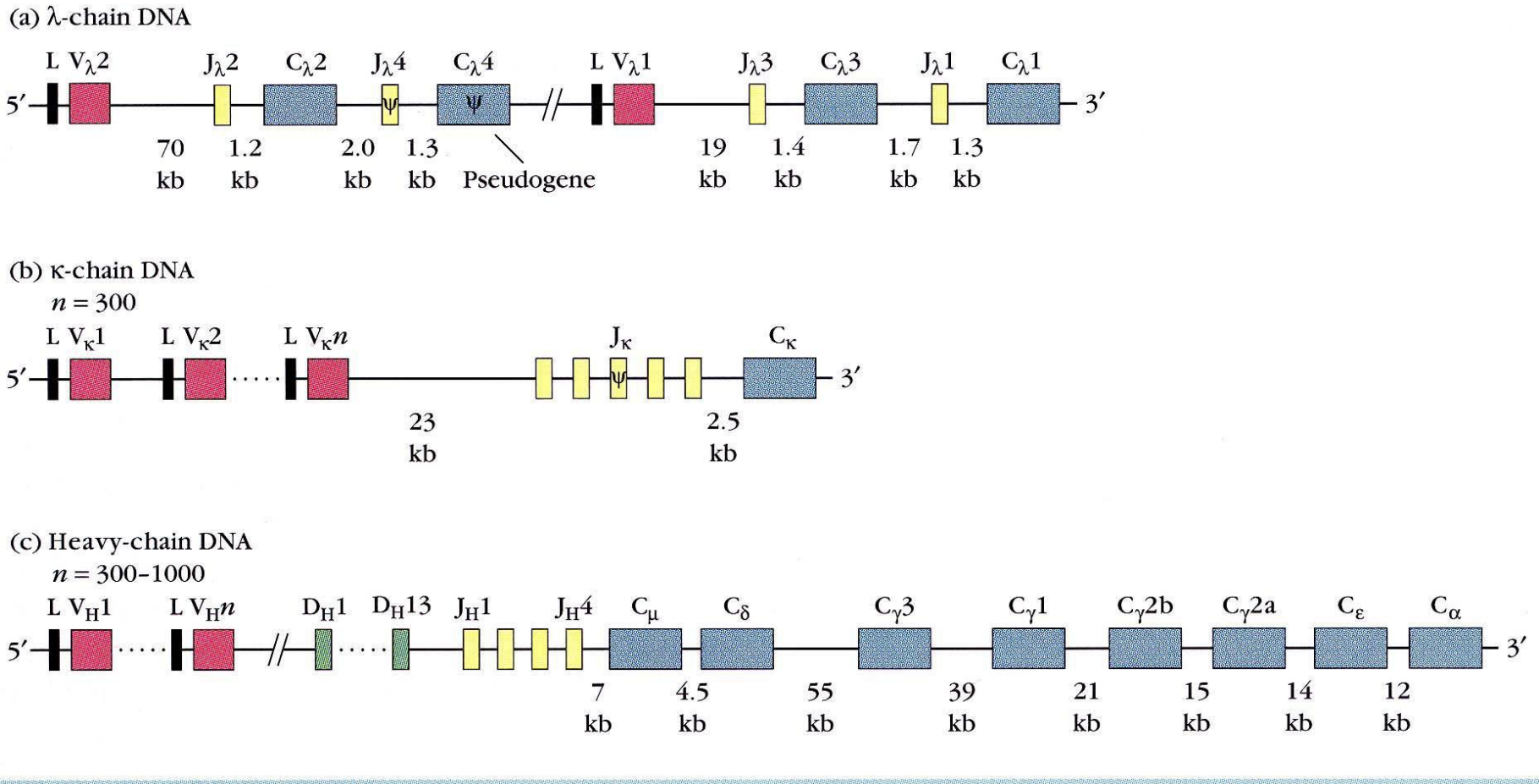


FIGURE 7-3

Organization of immunoglobulin germ-line gene segments in the mouse: (a) λ light chain, (b) κ light chain, and (c) heavy chain. The λ and κ light chains are encoded by V, J, and C gene segments. The heavy chain is encoded by V, D, J, and C gene segments. The distances in kilobases (kb) separating the various gene segments in mouse germ-line DNA are shown below each chain diagram.

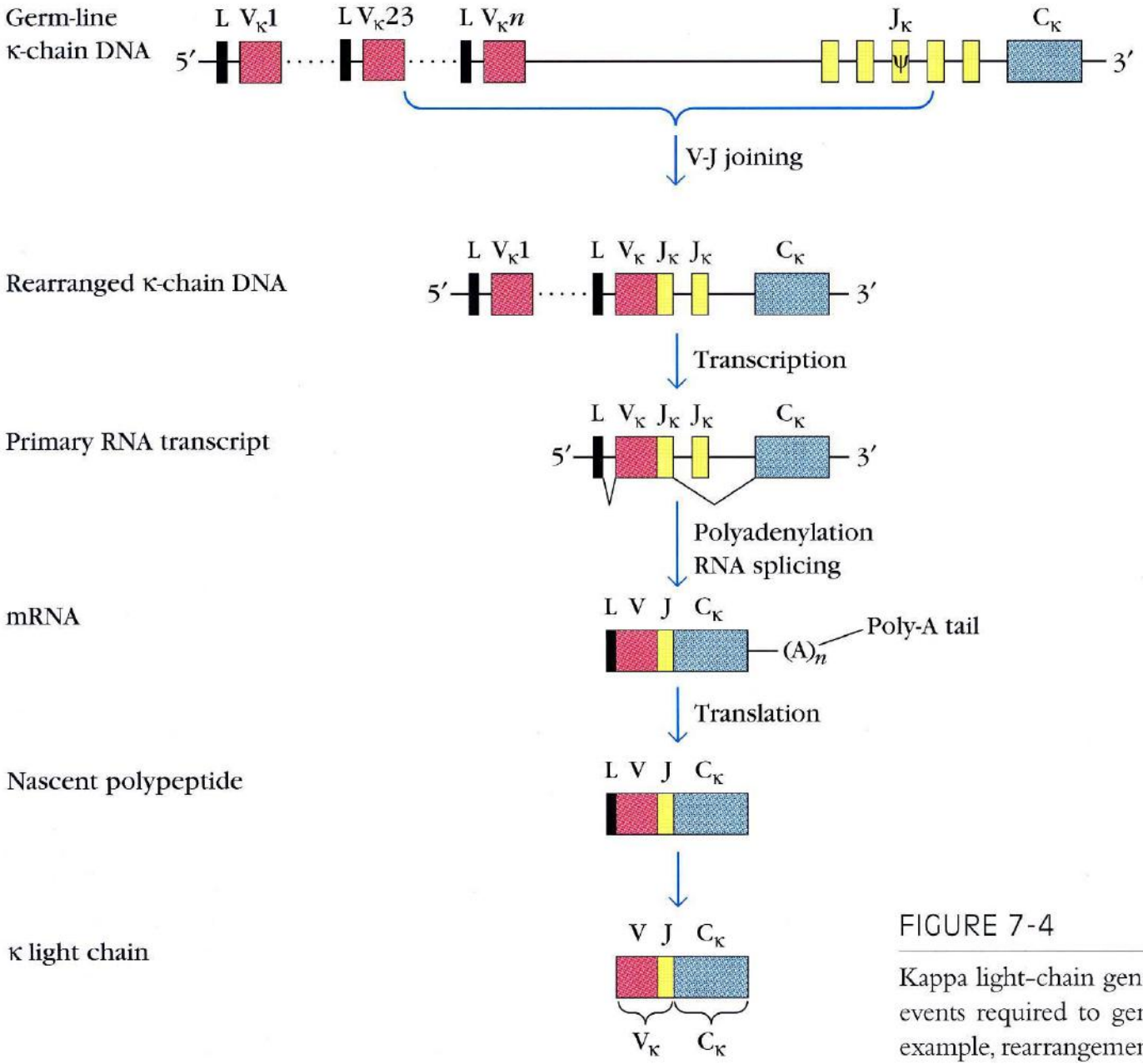


FIGURE 7-4

Kappa light-chain gene rearrangement and RNA processing events required to generate a κ light-chain protein. In this example, rearrangement involves joining of $V_\kappa 23$ and $J_\kappa 4$.

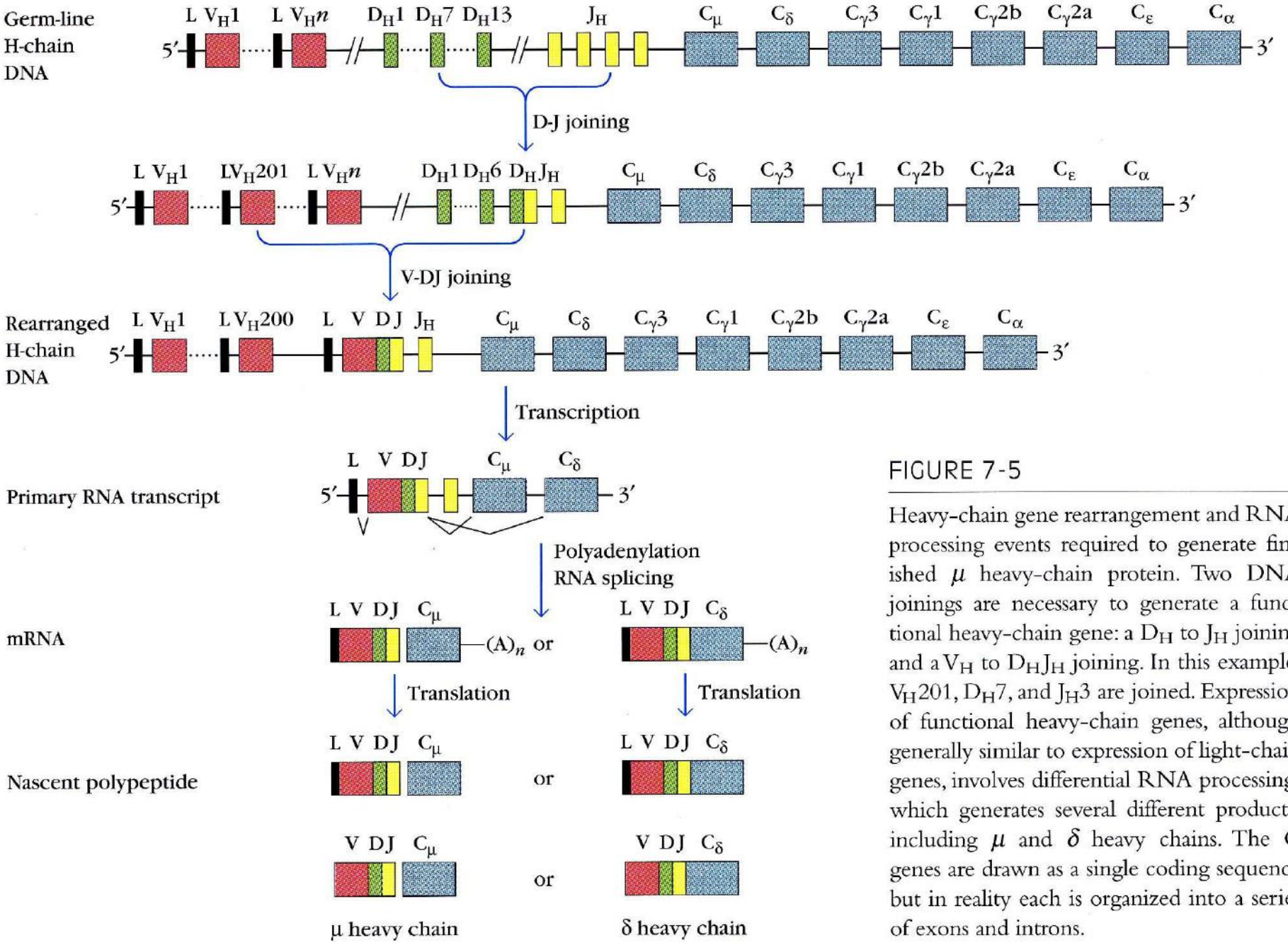


FIGURE 7-5

Heavy-chain gene rearrangement and RNA processing events required to generate finished μ heavy-chain protein. Two DNA joinings are necessary to generate a functional heavy-chain gene: a D_H to J_H joining and a V_H to D_HJ_H joining. In this example, V_H201, D_H7, and J_H3 are joined. Expression of functional heavy-chain genes, although generally similar to expression of light-chain genes, involves differential RNA processing, which generates several different products, including μ and δ heavy chains. The C genes are drawn as a single coding sequence but in reality each is organized into a series of exons and introns.

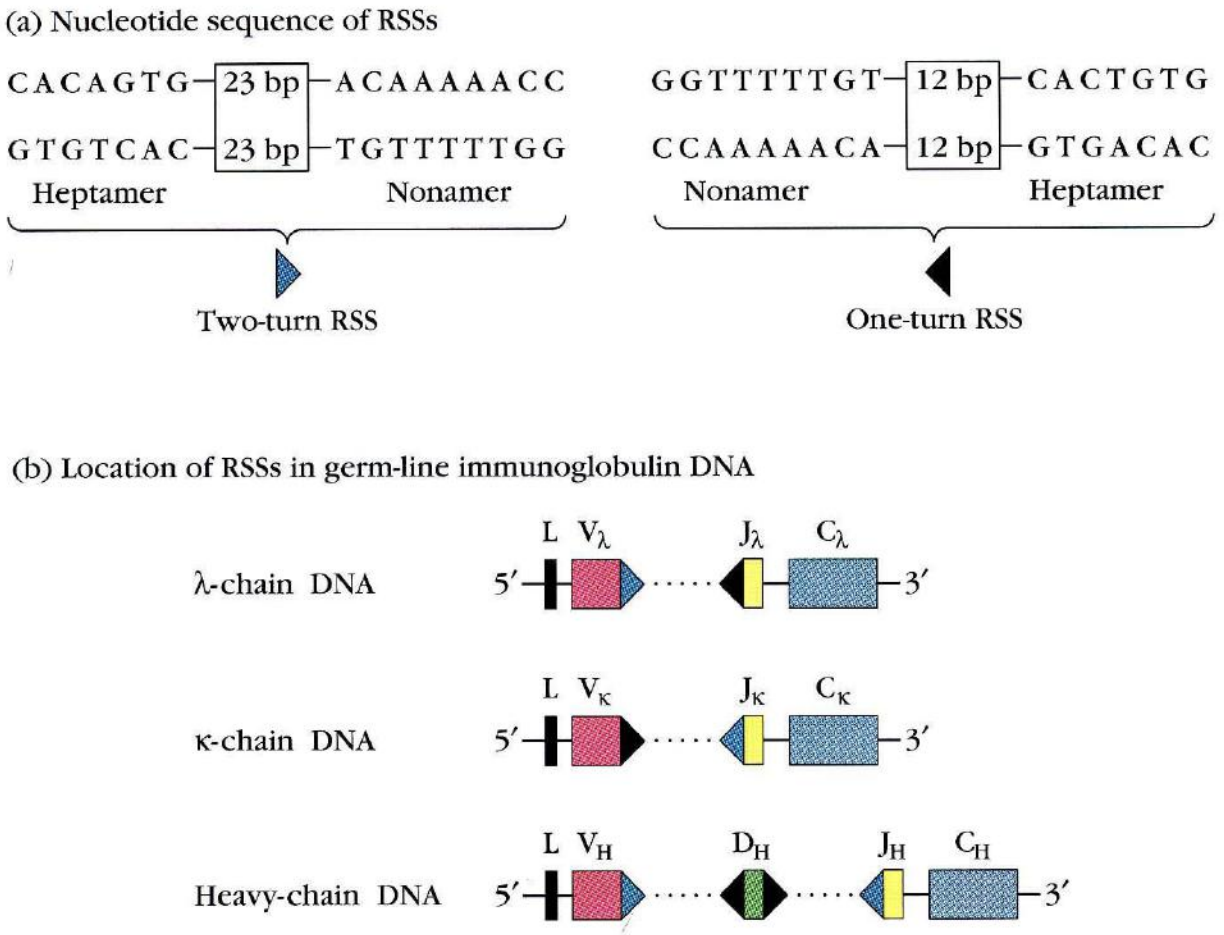


FIGURE 7-6

Two conserved sequences in light-chain and heavy-chain DNA function as recombination signal sequences (RSSs). (a) Both signal sequences consist of a conserved palindromic heptamer and conserved AT-rich nonamer; these are separated by nonconserved spacers of 12 or 23 base pairs. (b) The two types of RSS—designated one-turn RSS and two-turn RSS—have characteristic locations within λ-chain, κ-chain, and heavy-chain germ-line DNA. During DNA rearrangement, gene segments adjacent to the one-turn RSS can join only with segments adjacent to the two-turn RSS.

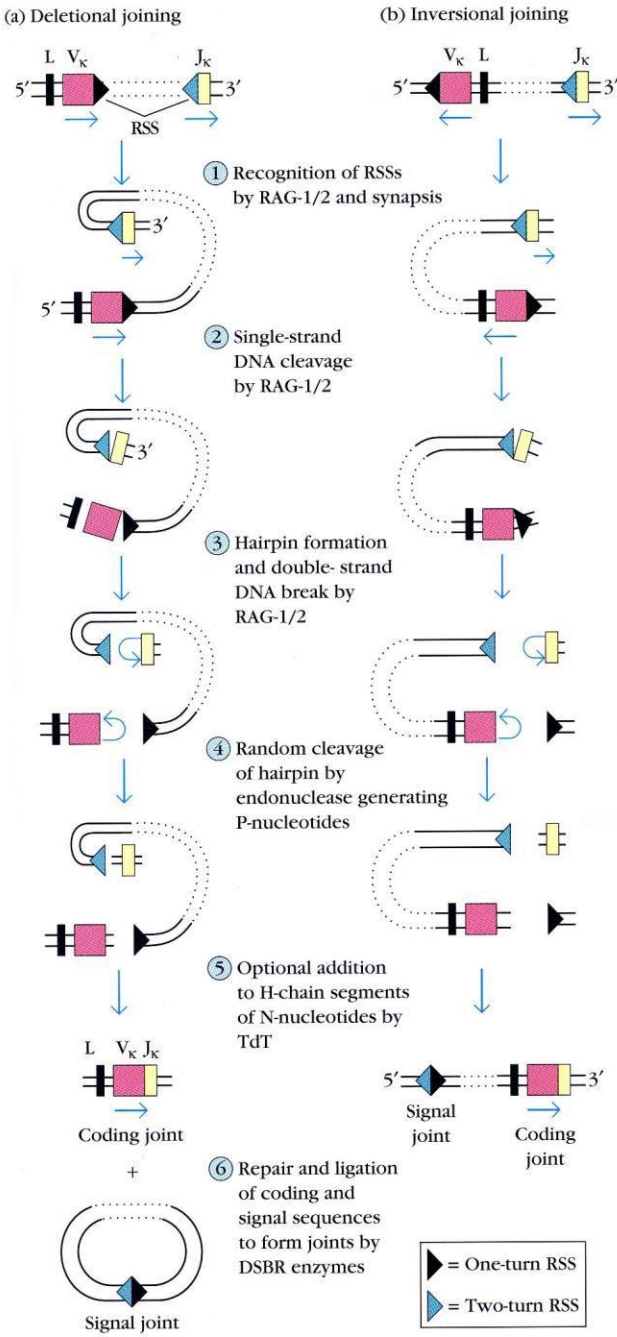


FIGURE 7-8

Model depicting the general process of recombination of immunoglobulin gene segments is illustrated with V_κ and J_κ. (a) Deletional joining occurs when the gene segments to be joined have the same transcriptional orientation (indicated by horizontal blue arrows). This process yields two products: a rearranged VJ unit that includes the coding joint and a circular excision product consisting of the recombination signal sequences (RSSs), signal joint, and intervening DNA. (b) Inversional joining occurs when the gene segments have opposite transcriptional orientations. In this case, the RSSs, signal joint, and intervening DNA are retained, and the orientation of one of the joined segments is inverted. In both types of recombination, a few nucleotides may be deleted from or added to the cut ends of the coding sequences before they are rejoined. See text for further discussion.

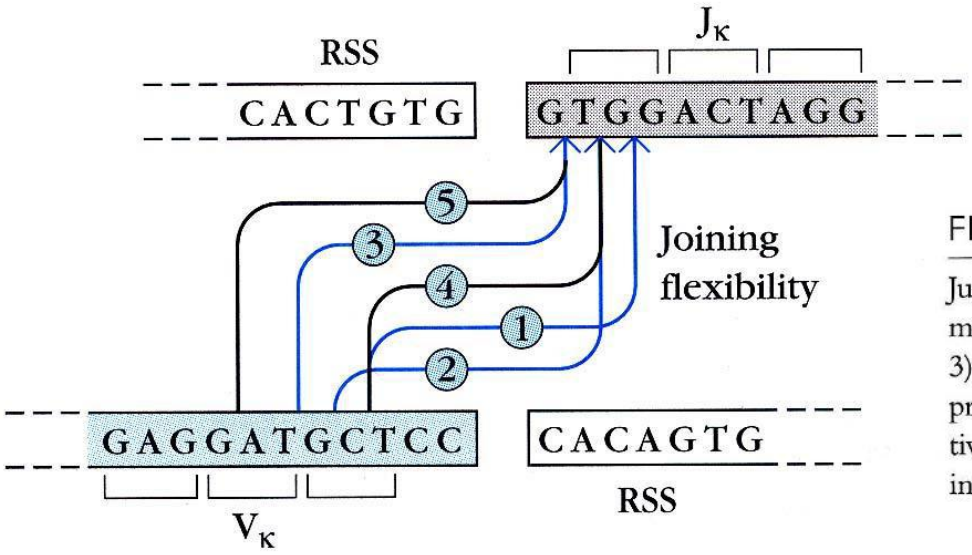


FIGURE 7-11

Junctional flexibility in the joining of immunoglobulin gene segments is illustrated with V_κ and J_κ. In-phase joining (arrows 1, 2, and 3) generates a productive rearrangement, which can be translated into protein. Out-of-phase joining (arrows 4 and 5) leads to a nonproductive rearrangement, which contains stop codons and is not translated into protein.

Productive rearrangements

1 Glu Asp Ala Thr Arg
GAGGATGCGACTAGG

2 Glu Asp Gly Thr Arg
GAGGATGGGACTAGG

3 Glu Asp Trp Thr Arg
GAGGATTGGACTAGG

Nonproductive rearrangements

4 Glu Asp Ala Asp Stop
GAGGATGCGGACTAG

5 Glu Val Asp Stop
GAGGTGGACTAG

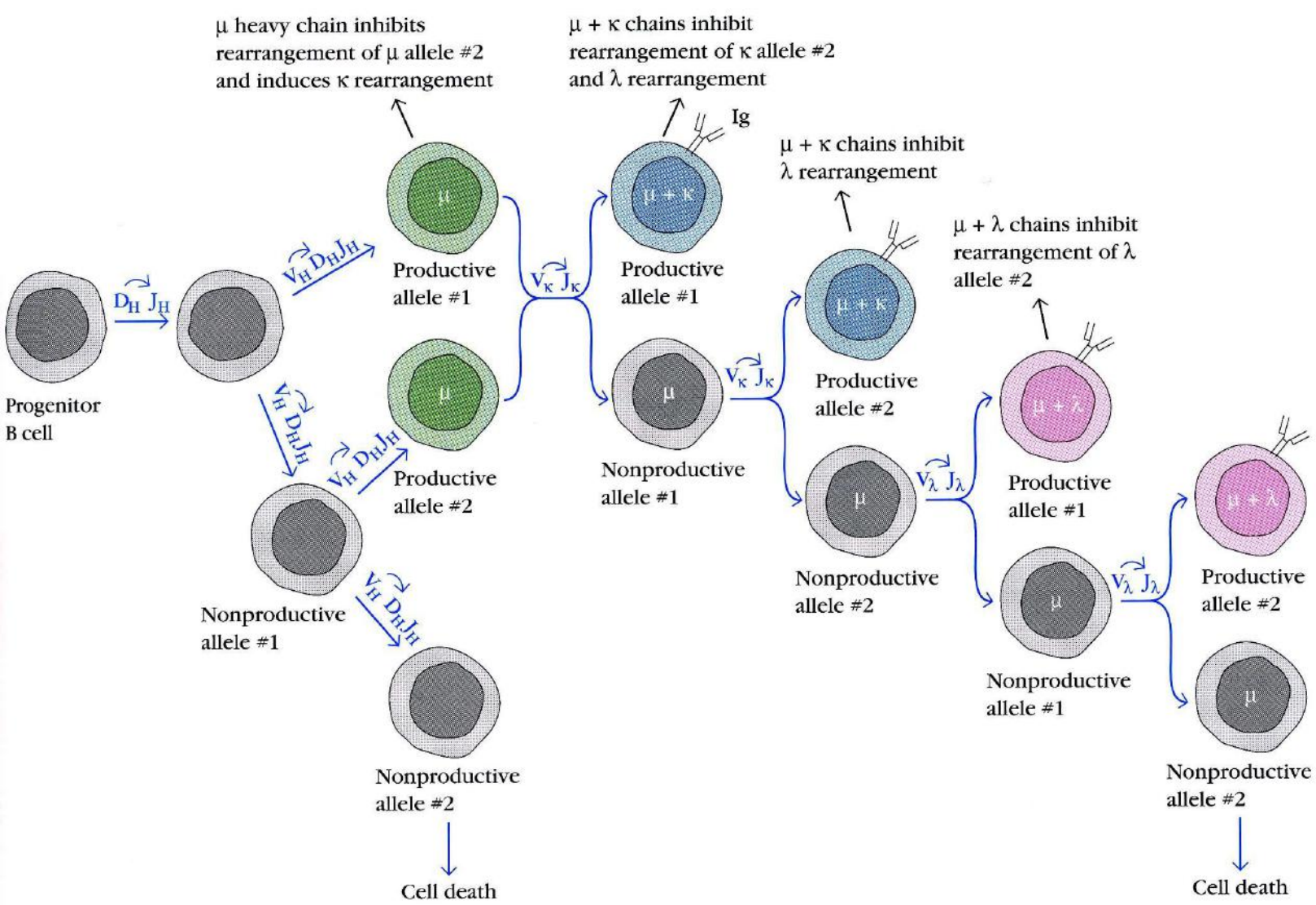


FIGURE 7-13

Model to account for allelic exclusion. Heavy-chain genes rearrange first, and once a productive heavy-chain gene rearrangement occurs, the μ protein product prevents rearrangement of the other heavy-chain allele and initiates light-chain gene rearrangement. In the mouse, rearrangement of κ light-chain genes precedes rearrangement of the λ genes, as shown here. In humans, however, either κ or λ rearrangement can proceed once a productive heavy-chain rearrangement has occurred. Formation of a complete immunoglobulin inhibits further light-chain gene rearrangement. If a nonproductive rearrangement occurs for one allele, then the cell attempts rearrangement of the other allele. [Adapted from G. D. Yancopoulos and F. W. Alt, 1986, *Annu. Rev. Immunol.* **4**:339.]

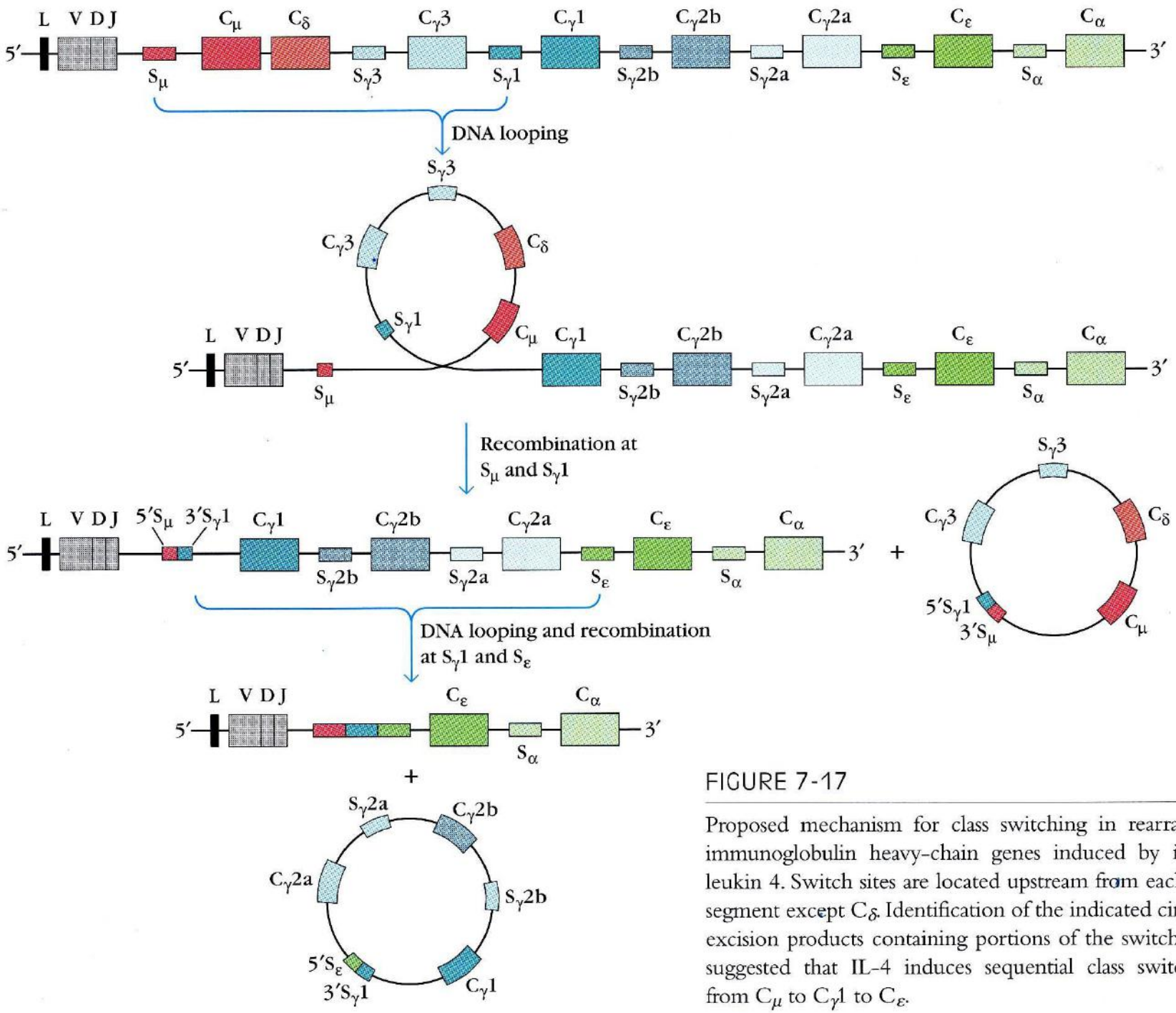


FIGURE 7-17

Proposed mechanism for class switching in rearranged immunoglobulin heavy-chain genes induced by interleukin 4. Switch sites are located upstream from each C_H segment except C_δ. Identification of the indicated circular excision products containing portions of the switch sites suggested that IL-4 induces sequential class switching from C_μ to $C_\gamma1$ to C_ε.

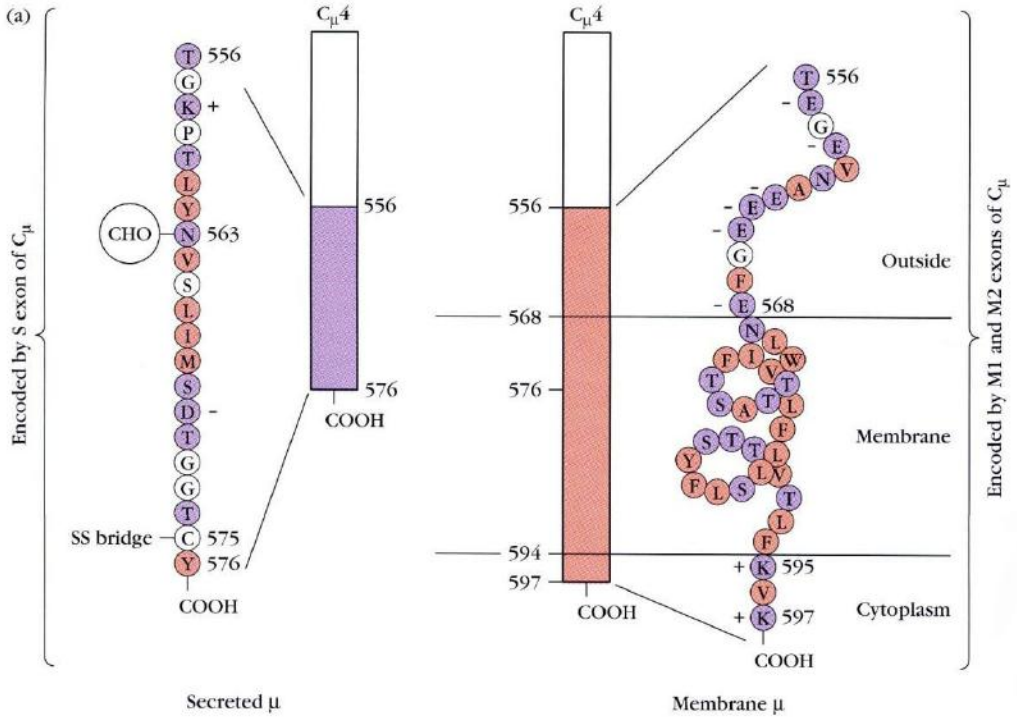
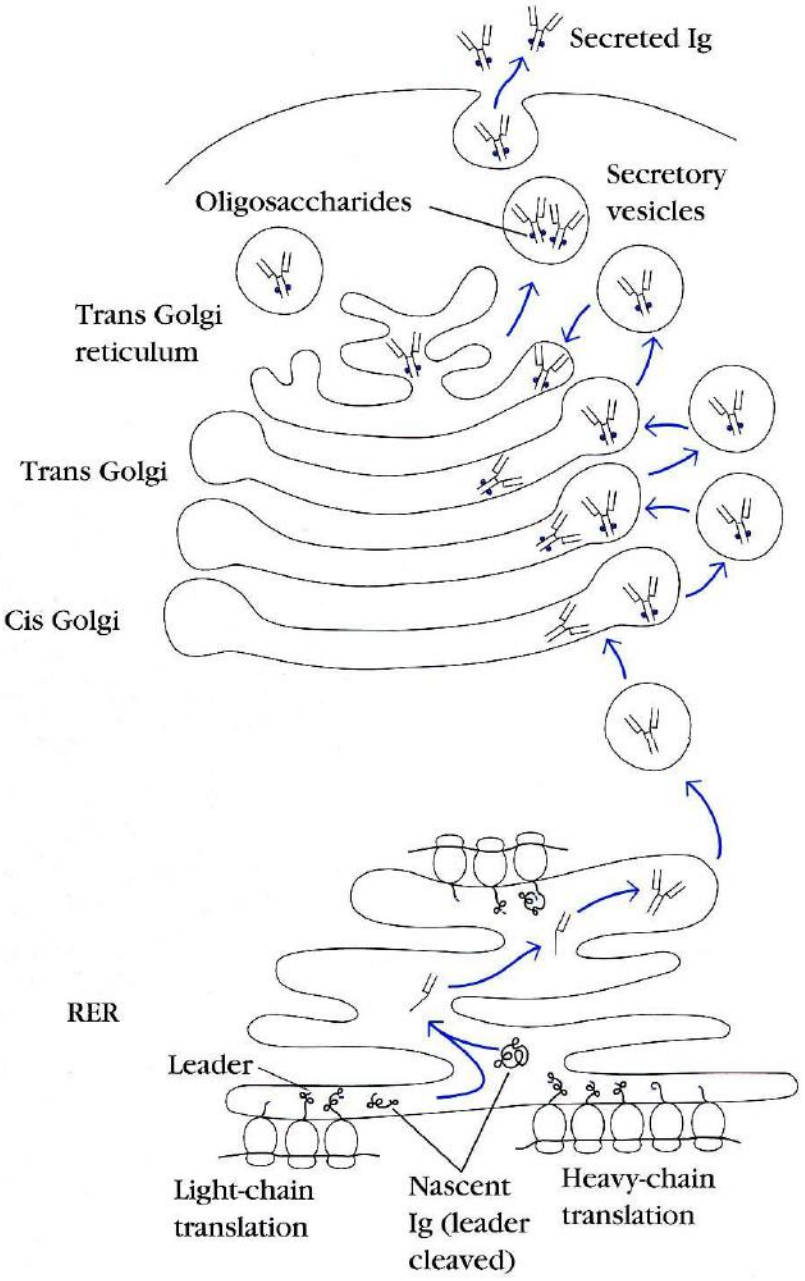


FIGURE 7-18

Expression of secreted and membrane forms of the heavy chain by alternative RNA processing. (a) Amino acid sequence of the carboxyl-terminal end of secreted and membrane μ heavy chains. Residues are indicated by the single-letter amino acid code. Hydrophilic residues and regions are shaded purple; hydrophobic residues and regions are shaded orange. Charged amino acids are indicated with a + or −. The rest of the sequence is identical in both forms. (b) Structure of the primary transcript of a rearranged heavy-chain gene showing the C_μ exons and poly-A sites. Polyadenylation of the primary transcript at either site 1 or site 2 and subsequent splicing (indicated by V-shaped lines) generates mRNAs encoding secreted or membrane μ chains.



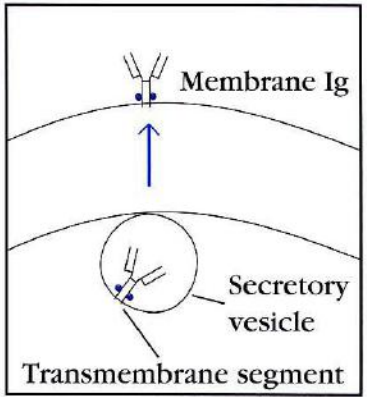


FIGURE 7-20

Synthesis, assembly, and secretion of the immunoglobulin molecule. The heavy and light chains are synthesized on separate polyribosomes (polysomes). The assembly of the chains to form the disulfide-linked and glycosylated immunoglobulin molecule occurs as the chains pass through the cisternae of the rough endoplasmic reticulum (RER) into the Golgi apparatus and then into secretory vesicles. The main figure depicts assembly of a secreted antibody. The inset depicts a membrane-bound antibody, which contains the carboxyl-terminal transmembrane segment. This form becomes anchored in the membrane of secretory vesicles and then is inserted into the cell membrane when the vesicle fuses with the membrane.

CUMULATIVE GENERATION OF MINIMUM ANTIBODY DIVERSITY IN THE MOUSE

MECHANISM OF DIVERSITY	HEAVY CHAIN	LIGHT CHAINS	
		κ	λ
ESTIMATED NUMBER OF SEGMENTS *			
Multiple germ-line gene segments:			
V	300–1000	300	2
D	13	0	0
J	4	4	3
POSSIBLE NUMBER OF COMBINATIONS †			
Combinatorial V-J and V-D-J joining	$300 \times 13 \times 4 = 1.6 \times 10^4$	$300 \times 4 = 1.2 \times 10^3$	$2 \times 3 = 6$
Junctional flexibility	+	+	+
P-region nucleotide addition	+	+	+
N-region nucleotide addition	+	–	–
Somatic mutation	+	+	+
Combinatorial association of heavy and light chains	$>1.6 \times 10^4 \times (>1.2 \times 10^3 + >6) = \gg 1.9 \times 10^7$		

* The estimated number of variable-region segments in human DNA is as follows: 100 V_H, 30 D_H, and 6 functional J_H; 100 V_κ and 5 J_κ; 100 V_λ, and 6 J_λ. The sources of antibody diversity in humans are identical to those in the mouse.

† (+) indicates mechanism makes a signficant contribution to diversity but to an unknown extent. (–) indicates mechanism does not operate.